

CLAIMS

1. A cell that expresses both chromosomal genes and extra-chromosomal genes, wherein (a) the expressed extra-chromosomal genes include a gene with an essential function, the expression of which is unconditionally required for survival of the cell, (b) the expressed chromosomal genes do not provide that essential function, and (c) the extra-chromosomal genes include a heterologous gene, the expression of which is controlled by a promoter that is functional in the cell.
2. A cell according to claim 1, where (d) the expressed extra-chromosomal genes comprise at least one further gene with a different essential function from (a), and (e) the expressed chromosomal genes also do not provide that essential function.
3. A cell according to claim 1 or claim 2, comprising at least one further extra-chromosomal heterologous gene, the expression of which is controlled by a promoter that is functional in the cell.
4. A method for expressing a heterologous gene, comprising the step of growing the cell of any one of claims 1-3 in a culture medium.
5. A method for purifying a protein, comprising the steps of: (a) growing the cell of any one of claims 1-3 by the method of claim 4, such that it expresses said protein; and (b) purifying the protein.
6. The method of claim 5, further comprising the step of: (c) treating the protein with a protease to provide a cleavage product of interest.
7. A cell that expresses both chromosomal genes and extra-chromosomal genes, wherein (a) the expressed extra-chromosomal genes include a gene with an essential function, the expression of which is unconditionally required for survival of the cell, (b) the expressed chromosomal genes do not provide that essential function, and (c) the extra-chromosomal genes include a conditionally-lethal gene, wherein the essential gene is *MOB1*, *Cdc33* or *Hsp10*.
8. A cell that expresses chromosomal genes, a first set of extra-chromosomal genes and a second set of extra-chromosomal genes, wherein (a) the expressed first and second sets of extra-chromosomal genes both include a gene with the same essential function, the expression of which is unconditionally required for survival of the cell, (b) the expressed chromosomal genes do not provide that essential function, (c) the first set of extra-chromosomal genes includes a conditionally-lethal gene, and (d) the second set of extra-chromosomal genes includes both a conditionally-required gene and a heterologous gene.
9. A cell according to claim 8, wherein (e) the cell also expresses a third set of extra-chromosomal genes comprising a gene with a different essential function to that of the gene found in both the

first and second set of extra-chromosomal genes, the expression of which is required for survival of the cell, (f) a conditionally required gene and a heterologous gene.

10. An extra-chromosomal vector, comprising: (a) an essential gene whose expression is unconditionally required for survival of a cell of interest; (b) a conditionally-required gene to allow selection of host cells which include the extra-chromosomal vector; and (c) a gene encoding a heterologous protein of interest operably linked to a promoter that is functional in the cell of interest.
11. The vector of claim 10, wherein the vector is a plasmid.
12. The vector of claim 10 or claim 11, wherein the conditionally-required gene is a resistance gene.
13. The vector of claim 12, wherein the resistance gene is an antibiotic resistance gene, a drug resistance gene, or a herbicide resistance gene.
14. The vector of claim 10 or claim 11, wherein the conditionally-required gene complements an auxotrophic mutation in the host's chromosome.
15. An extra-chromosomal vector, comprising: (a) an essential gene whose expression is unconditionally required for survival of a cell of interest; (b) a conditionally-lethal gene to allow selective killing of host cells which include the extra-chromosomal vector, wherein the essential gene is *MOB1*, *Cdc33* or *Hsp10*.
16. The vector of any one of claims 10 to 15, comprising one or more of the following elements: (i) an origin of replication functional in a host cell of interest; (ii) a polylinker containing a plurality of restriction sites; (iii) a transcription termination sequence downstream of one or more of the promoters and their coding sequences in the vector.
17. The vector of any one of claims 10 to 16, comprising one or more of: (iii) an origin of replication functional in bacteria; and (iv) an antibiotic resistance marker suitable for selection of bacterial transformants.
18. A method for preparing the cell of any one of claims 1-3, comprising the steps of: (a) obtaining the cell of claim 8, which includes a conditionally-lethal gene(s); (b) transforming the cell with the vector of any one of claims 10, 11, 12, 13, 14, 16 or 17, which includes a conditionally-required gene(s), to give the cell of claim 8; (c) selecting transformants which express the vector's conditionally-required gene(s); and (d) selecting transformants which lose the conditionally-lethal gene(s).
19. The cell, method or vector of any preceding claim, wherein the essential gene is a gene whose loss prevents cell division, prevents mitosis, prevents transcription, or prevents translation.

20. The cell, method or vector of any preceding claim, wherein the essential gene has a coding sequence of <3000 base pairs.
21. The cell, method or vector of any preceding claim, wherein the essential gene is not lethal when hyper-expressed.
- 5 22. The cell, method or vector of any preceding claim, wherein the essential gene is *MOB1*.
23. The cell, method or vector of any preceding claim, wherein the heterologous gene comprises a sequence from a higher eukaryote or a eukaryotic virus.
24. The cell, method or vector of claim 23, wherein the eukaryote is an animal.
25. The cell, method or vector of any preceding claim, wherein the heterologous gene encodes a
10 fusion protein comprising a first sequence and a second sequence.
26. The cell, method or vector of claim 25, wherein the junction between the first sequence and second sequence includes a protease recognition sequence.
27. The cell, method or vector of claim 26, wherein the protease is thrombin, factor Xa protease, enterokinase, endopeptidase rTEV or human rhinovirus protease 3C.
- 15 28. The cell, method or vector of claim 25, wherein the junction between the first sequence and second sequence includes an intein.
29. The cell, method or vector of any preceding claim, wherein the heterologous gene comprises a sequence encoding glutathione-S-transferase, a poly-histidine tag, a calmodulin-binding peptide, a maltose-binding protein, a chitin-binding domain, or an immunoaffinity epitope.
- 20 30. The cell, method or vector of any preceding claim, wherein the heterologous gene encodes a protein which forms oligomers.
31. The cell, method or vector of any preceding claim, wherein the heterologous gene is expressed as a soluble protein.
32. The cell, method or vector of any preceding claim, wherein expression of the essential gene is
25 controlled by an inducible promoter.
33. The cell, method or vector of any preceding claim, wherein expression of the heterologous gene is controlled by an inducible promoter.
34. The cell, method or vector of claim 32 or claim 33, wherein the promoter is a repressible promoter.
- 30 35. The cell, method or vector of claim 34, wherein the heterologous gene and the essential gene are inducible and/or repressible by the same stimulus.

36. The cell, method or vector of any preceding claim, wherein expression of the essential gene and/or the heterologous gene is controlled by a galactokinase/UDP-glucose 4 epimerase promoter.
37. The cell, method or vector of any preceding claim, wherein the cell is a eukaryote.
38. The cell, method or vector of claim 37, wherein the eukaryote is a yeast.
- 5 39. The cell, method or vector of claim 38, wherein the yeast is *Saccharomyces cerevisiae* or *Schizosaccharomyces pombe*.
40. The cell, method or vector of any preceding claim, wherein the heterologous gene encodes a Ltel protein, a Bfal protein, a Bub2 protein, a CDC5 protein, a CDC15 protein, a CDC28 protein, a Tpl2 protein, a SARS virus Nsp13 protein, or a mRNA Cap1 methyl transferase protein.